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Review

Monthly Focus: Cardiovascular & Renal

Therapeutic potential of Na-H exchange inhibitors for the treatment of heart failure

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The Na-H exchanger (NHE) represents a family of transporters which regulate intracellular pH by removing protons in exchange for sodium influx in an electroneutral 1:1 stoichiometric relationship. Six isoforms have thus far been identified with the NHE-1 subtype representing the primary isoform in the cardiac cell. It is well-established that NHE-1 contributes to cardiac injury produced by ischaemia and reperfusion and inhibitors of the antiporter exert excellent cardioprotection. More recent evidence suggests that NHE-1 may also be important for cell growth and may contribute to the maladaptive remodelling which contributes to heart failure particularly the early hypertrophic responses. Evidence from *in vitro* studies suggest that NHE-1 inhibitors attenuate cardiomyocyte hypertrophy in response to various stimuli whereas *in vivo* studies report substantial attenuation of both hypertrophy and heart failure by these agents, especially after myocardial infarction. Accordingly, NHE-1 inhibitors could emerge as important therapeutic tools for the attenuation and treatment of heart failure.

Keywords: *cariporide, hypertrophy, myocardial remodelling, NHE-1*

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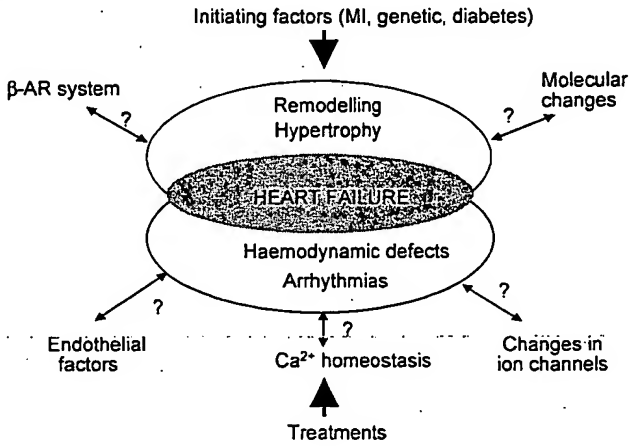
1. Introduction

Heart failure is an important and complex clinical syndrome in which cardiac output is insufficient to meet the body's homeostatic requirements. However, in reality heart failure is difficult to define because of its complexity which is much more than the inability of the heart to pump blood efficiently. Rather, as shown in **Figure 1**, it is now evident that heart failure is not a disease *per se* but rather the final common pathway for numerous cellular and molecular defects caused by many instigating factors including myocardial infarction, genetic factors, diabetes and pulmonary hypertension. The incidence of congestive heart failure is rising tremendously due to an ageing population coupled with improved survival rates after myocardial infarction, since these patients are at risk to develop heart failure. Indeed, although heart failure can result from various causes, myocardial infarction represents the primary cause of heart failure in developed countries [1]. From 1979 to 1997, death rates from heart failure increased by almost 130%. In the US almost 5,000,000 individuals have heart failure with more than 500,000 new cases divided equally between males and females, diagnosed each year. The five year mortality rate for heart failure is about 50%. These patients exhibit an incidence of sudden cardiac death at 6 - 9 times the rate of the general population. Overall, the

Figure 1: Diagram showing some of the inter-relationships between factors which may contribute to heart failure, reinforcing the concept that heart failure represents a central complex syndrome which is manifested by a large number of phenotypic and genotypic changes. β-AR: β-adrenoceptor.



emotional, physical and financial burden of heart failure for individuals and society in general is staggering.

Recent advances in molecular and cellular biology have affirmed that heart failure extends beyond abnormal heart function and organ physiology but rather involves numerous intracellular defects. Moreover, the complexity of the heart failure process is well-known, particularly in view of the numerous cellular and molecular changes that are seen in heart failure many of which appear to be inter-related (**Figure 1**). To better understand the underlying basis for heart failure there are two critical components of the heart failure process which need to be noted. The first represents the initial adaptive hypertrophic response which follows myocardial injury and the second representing the eventual evolution to heart failure [2]. It is indeed well accepted that attenuation of the early adaptive hypertrophy is particularly critical since this can result an attenuation of the heart failure response. As such, understanding the hypertrophic response represents a pivotal component of our overall appreciation of the complexity of the heart failure process.

2. Current drug treatment of heart failure

Pharmacological treatment of heart failure involves drugs which act on the circulatory system such as diuretics and vasodilators, those directly targeting the myocardium such as positive inotropic agents or drugs which alter neurohumoral system including ACE inhibitors, or aldosterone or β-adrenoceptor blockers [1]. Recent emphasis has been placed on the development of strategies directed at slowing the disease process by direct actions on the cardiac myocytes. Indeed, the beneficial effects of a number of strategies previously thought to reflect vasodilating effects, such as ACE inhibitors, may actually involve attenuation of the remodelling process. This reflects the fact that many of the endogenous vasoactive substances also produce direct effects on the myocardial cell *via* interaction with distinct cell receptor subtypes. Indeed, many of these factors including endothelin-1 (ET-1) or angiotensin II can be produced within the cardiomyocyte thus acting as autocrine factors. The salutary effects of β-adrenoceptor blockade, may also involve a direct 'antiremodelling' action. Thus, many newer experimental approaches for heart failure, such as angiotensin (AT_1), ET-1 or cytokine receptor blockade are aimed at slowing progression of the disease thereby improving survival.

3. Cardiac hypertrophy: a key contributor to heart failure and potential target for NHE-1 inhibition

Conditions which produce an increase in ventricular wall tension or tissue stress, or the direct effects of hormones, autocrine or paracrine factors, can all result in adaptive responses manifested by hypertrophy. The latter represents an initial adaptive response which temporarily contributes towards

improved cardiac performance. Thus, the early hypertrophic response is one of the major compensatory responses to myocardial injury [2]. However, prolonged cardiac hypertrophy is well-established as a major contributor to the heart failure process and indeed the degree of hypertrophy is a predictor of poor prognosis in patients not only with heart failure [3] but also cardiac disease in general [4]. Reversal of the hypertrophic process therefore represents an important therapeutic challenge for the treatment of heart failure [5]. Extensive research is ongoing which attempts to define the molecular mechanisms that contribute to or underlie cardiac hypertrophy [6]. These mechanisms generally involve various signal transduction pathways, among which include various kinases such as mitogen-activated protein (MAP) kinase and protein kinase C (PKC). Of relevance to the present discussion and as will be alluded to in more detail below, a large number of hormonal paracrine and autocrine factors which stimulate cell signalling pathways, also activate the NHE, which is the major mechanism for regulating intracellular pH in the cardiac cell after acidosis [7-9]. Such factors include angiotensin II, ET-1 and α_1-adrenergic agonists and which will be discussed below. It should be noted that inhibition of NHE has been extensively demonstrated to attenuate myocardial injury produced by ischaemia and reperfusion. However, recent evidence for its role in the development of myocardial hypertrophy and subsequent development of heart failure appear to involve mechanisms unrelated to its role in ischaemia- or reperfusion-induced cardiac injury. Accordingly, it is evident that the hypertrophic response is complex and multifactorial requiring the interplay between a number of intracellular factors potentially acting in concert. Despite the current lack of knowledge regarding the precise mechanisms of hypertrophy, it is critical to underline that hypertrophy contributes to cardiac dysfunction through a multiplicity of causes including defective energy metabolism and oxygen delivery, reduced coronary perfusion because of increased extravascular compression, as well as an increased incidence of arrhythmias. In addition, as already noted, hypertrophy progresses and appears to be a prerequisite for evolution to heart failure. Thus, prevention of hypertrophy, or as has become evident recently, its reversal, represent important therapeutic goals for the treatment of heart failure.

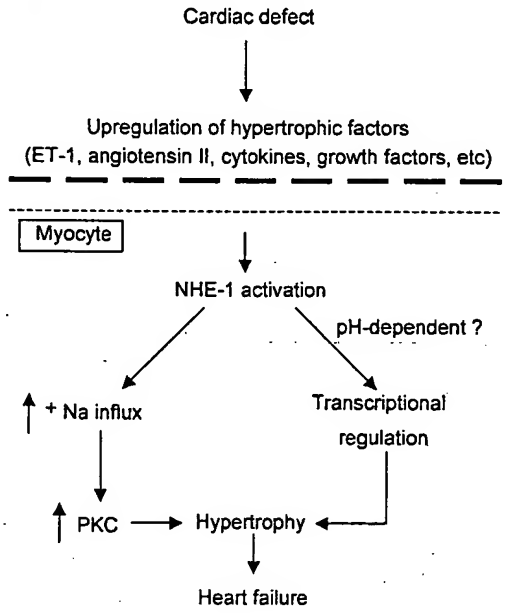
4. The myocardial Na-H exchanger: a brief synopsis

Changes in intracellular pH can have profound effects on cardiac contractility through complex mechanisms and as such it is critical that the cell possesses mechanisms by which intracellular pH is regulated, especially after intracellular acidosis as a consequence of myocardial ischaemia. Two major alkalinising exchangers exist in the cardiac cell including the NHE and a Na/HCO_3 symport. NHE represents one of the key mechanisms for restoring intracellular pH following ischaemia-induced acidosis by extruding protons concomitantly with Na^+ influx. The simultaneous entry of Na^+ during NHE activation indicates that this process is also an important route for increasing intracellular Na^+ concentrations during various conditions. Thus far six isoforms of the exchanger have been identified although the heart contains the ubiquitous subtype 1 (NHE-1), a glycoprotein having a molecular weight of approximately 110 kDa. Detailed discussion concerning NHE-1 structure and regulation can be found in recent reviews from the author's laboratory [10,11].

4.1 NHE-1 inhibition and protection of the ischaemic and reperfused myocardium

It is now well-established that NHE-1 is activated during ischaemic and reperfusion and this activation contributes to cell injury under those conditions. Moreover, inhibitors of NHE-1 activity are very effective cardioprotective agents as has been demonstrated under a large variety of experimental conditions and with numerous species (for review see [10,11]). This has led to extensive development of novel NHE-1 specific inhibitors with potential as cardiovascular therapeutic agents. One such inhibitor, cariporide, has recently undergone clinical evaluation in a large international multicentre trial in patients with acute coronary syndromes [12]. Thus, this area represents a potentially attractive area of research which holds promise for the development of novel therapeutic strategies for treatment of patients with coronary artery disease. This topic has received extensive coverage over the past number of years and readers are referred to a number of review articles which have covered the area of NHE involvement in ischaemic heart disease and clinical development of NHE-1 specific inhibitors [10,13,14].

Figure 2: Simplified schematic illustrating the potential role of NHE-1 in the adaptive response to injury. Many hypertrophic factors upregulated by defective cardiac function are potent NHE-1 activators and as such their hypertrophic effects may be mediated by NHE-1 through an as yet unresolved mechanism.



5. Rationale for NHE-1 involvement in hypertrophy and heart failure

There are a number of lines of evidence suggesting that NHE-1 may represent a key factor mediating hypertrophic responses, especially after myocardial infarction and thus suggesting that the exchanger could be an important cellular target for attenuation of both the hypertrophic responses as well as heart failure. From a theoretical perspective, it is important to indicate that NHE-1 stimulation can occur through receptor-dependent mechanisms. This reflects the fact that the antiporter is the target of multiple signalling pathways such as those activated by various kinases and G protein-coupled receptors [9,10]. The intracellular pathways leading to activation of NHE-1 are not well understood. Increasing evidence suggests that NHE-1 activation through signalling mechanisms is dependent on MAP kinases (MAPK) especially in response to growth factors which are potential candidates as hypertrophic agents. Indeed, NHE-1 possesses consensus sequences for MAPK and various studies have implicated MAPK in NHE-1 phosphorylation and activation [15]. Recently a role for p90rsk in ET-1-induced MAPK-dependent phosphorylation of NHE-1 has been demonstrated in rat myocardium [16]. This system may be of critical importance in hypertrophy and heart failure and may contribute to the well-known ability of ET-1 to produce hypertrophy and participate in the heart failure process. Other endogenous factors which activate NHE-1 most likely by similar cell signalling mechanisms and which are important for the development of cardiac hypertrophy and heart failure include angiotensin II and α_1-adrenergic agonists [10,11]. Hence, there appears to be a good relationship between hypertrophic factors and their ability to activate NHE-1. **Figure 2** briefly summarises the potential pivotal role for NHE-1 in mediating hypertrophy and heart failure. Potential mechanisms are discussed in section 5.4.

5.1 Experimental evidence for NHE involvement in cell growth of non-cardiac tissue

In addition to a strong theoretical basis for NHE involvement in cell growth, there is now compelling experimental evidence from studies using a variety of tissues that cell growth and proliferation may be regulated by NHE and that NHE inhibitors can block such responses. Early evidence for NHE involvement in cell growth came from studies using non-cardiac proliferating cell types. For example, it was shown that neointimal thickening and smooth muscle cell proliferation after carotid artery balloon injury can be

markedly inhibited by NHE inhibitors [17,18]. Moreover, angiotensin II-induced vascular smooth muscle cell hypertrophy is reduced by NHE inhibition [19]. Thus, in this context NHE inhibitors could be potentially useful for prevention of atherosclerotic lesion development and restenosis or indeed many vascular conditions where cell proliferation and hypertrophy appear to represent contributing factors.

5.2 Experimental evidence for NHE-1 involvement in cardiac cell hypertrophy

The underlying basis for NHE involvement in cardiac hypertrophy is likely identical as that suggested for other cell types where the antiporter plays an important role in protein synthesis through an as yet not fully understood pathway. It is important to re-emphasise that NHE, with respect to the cardiac cell NHE-1, represents a key downstream factor activated by a variety of hypertrophic stimuli, a property which is not likely to be shared by any other cellular regulatory process. The ability of a large number of hypertrophic stimuli to activate NHE-1 does not necessarily confirm or prove cause and effect relationships. As discussed below this concept has, however been reinforced primarily in studies which have utilised pharmacological inhibitors of the antiporter, especially drugs such as cariporide (HOE 642) which demonstrate marked selectivity and specificity for NHE-1 and are generally devoid of non-specific effects [20]. It should also be noted that in addition to activation of NHE-1 by various hypertrophic stimuli, increased gene expression of the antiporter can also occur as has been demonstrated in pressure-overloaded hearts [21].

A number of investigators have used cultured neonatal cardiac myocytes or isolated tissues to demonstrate NHE-1 involvement in hypertrophy. These *in vitro* approaches are advantageous in that one can study precisely the direct hypertrophic responses to relevant stimuli in the absence of other contributing factors. Moreover, the study of the cellular and molecular basis for hypertrophy is facilitated by these types of investigations. The primary limitation of using *in vitro* methodology is that the complete picture of the complex underlying mechanisms contributing to both the hypertrophy and the subsequent development of heart failure, cannot be fully addressed. In addition, the use of neonatal cells could be problematic, and results using such cells should be interpreted cautiously since it is possible that neonatal cells respond differently to various stimuli compared with adult ventricular myocytes. Nonetheless, the use of cultured neonatal cardiac myocytes has provided useful and important information for understanding mechanisms of hypertrophic responses.

As noted above, studies using cardiac cells have consistently demonstrated that NHE inhibitors block hypertrophic responses to various stimuli. For example, stretch-induced stimulation in protein synthesis in neonatal cardiac myocytes as well as stretch-induced alkalinisation in feline papillary muscles can be blocked by NHE inhibitors [22,23] as can noradrenaline-induced protein synthesis in cultured rat cardiomyocytes [24]. The fact that NHE inhibitors can block the hypertrophic response to a wide variety of stimuli, is strongly suggestive of the antiporter as a common key downstream mediator.

5.3 Experimental evidence for NHE involvement in heart failure

Research into the potential role of NHE, or more specifically, NHE-1, in the development of heart failure have utilised *in vivo* approaches and well-defined heart failure models. Initial experiments in this area have utilised relatively non-specific inhibitors of the antiporter such as amiloride. Indeed, it has been shown that orally-administered amiloride reduces fibre diameter in both the rat coronary ligation [25] and murine dilated cardiomyopathy models [26]. The chronic rat coronary artery ligation model is of particular usefulness in that it is characterised in a well-defined series of post-infarction adaptive responses culminating in heart failure similar to that seen in the clinical setting. Current therapy for heart failure such as the use of ACE inhibitors, have been developed using this particular experimental model and approach. Our laboratory has utilised this model to identify potential beneficial effects of cariporide, the NHE-1 selective inhibitor, on both early and late postinfarction-induced heart failure. Orally administered cariporide completely abrogated the increased length of surviving myocytes one week after coronary artery occlusion and improved contractile dysfunction [27]. It is important to note that these effects occurred in the absence of afterload reduction. Moreover, improved haemodynamics were associated with an almost complete abrogation or left ventricular hypertrophy.

We further expanded these studies to a more chronic (three month) follow-up period where both adaptive, i.e., hypertrophic and heart failure responses are more pronounced. As with the one week study, cariporide significantly attenuated left ventricular dysfunction which included a marked attenuation of left ventricular end-diastolic pressures [28]. Thus, both systolic and diastolic dysfunction are reduced by NHE-1 inhibition. **Figure 3** summarises some of the haemodynamic changes in the three month postinfarcted rat and the effect of cariporide. Cariporide also significantly reduced the degree of LV dilation, although this effect was seen primarily in animals with large infarcts. Hypertrophy, including heart weights and cell dimensions were reduced by cariporide by approximately 50% and shortening of surviving myocytes was completely preserved. Infarct size was unaffected in the one-week or the three-month study strongly reinforcing the concept that the beneficial effects of cariporide are unrelated to a direct cytoprotective action but instead reflect the drugs' ability to decrease the hypertrophic/remodelling process.

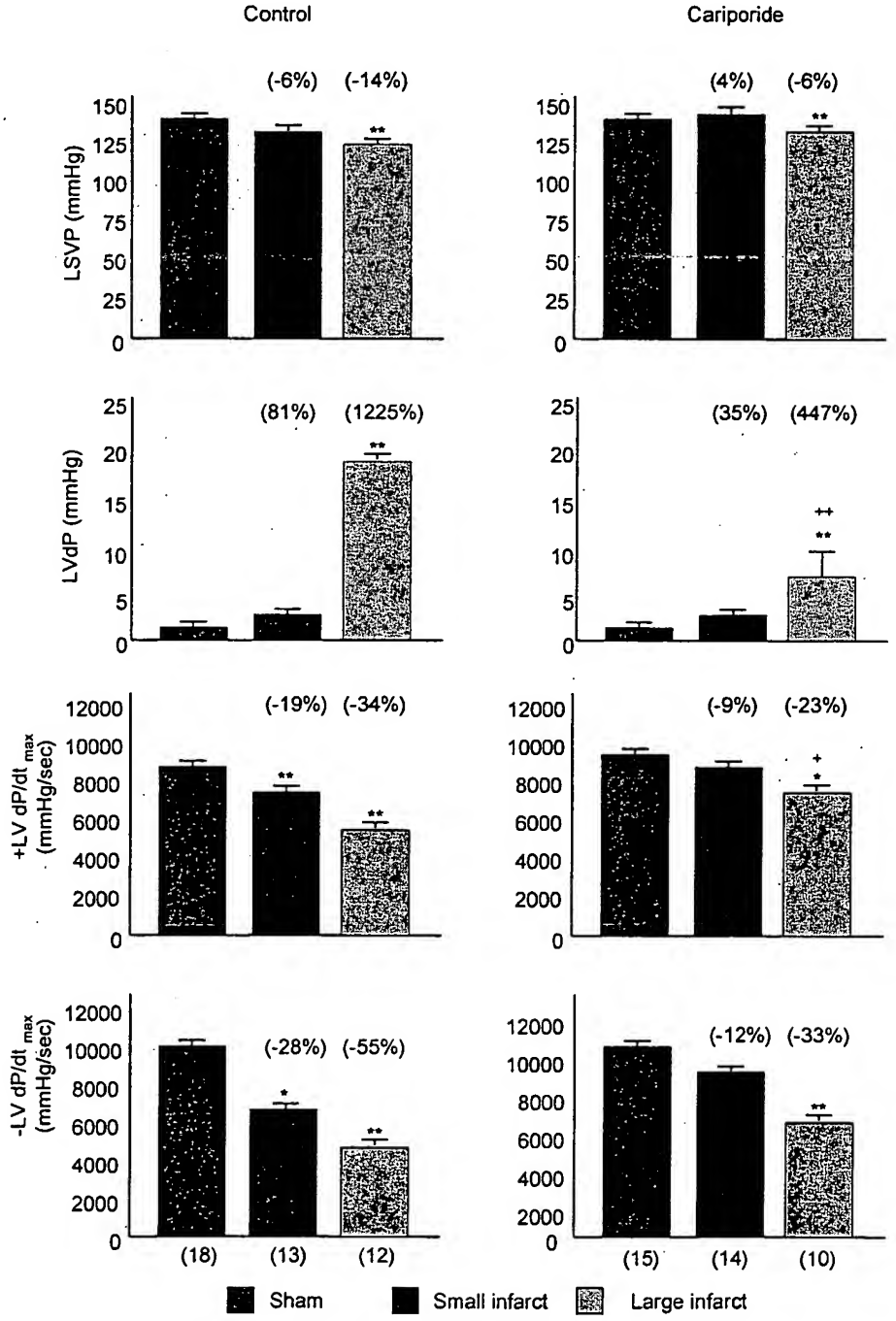
It is likely that the importance of NHE-1 to heart failure may not be restricted only to postinfarction responses. In this regard, we have recently observed that in a rat model of right ventricular hypertrophy and heart failure secondary to pulmonary artery injury, cariporide significantly attenuates right ventricular compensatory responses in the absence of pulmonary effects, thus suggesting a direct effect on right ventricular remodelling processes by the NHE-1 inhibitor [29].

5.4 Possible mechanisms

Although experimental evidence strongly implicates NHE-1 in heart failure, particularly in terms of regulating the initial adaptive hypertrophic and remodelling processes, the precise mechanism for NHE-1 involvement remains to be elucidated and remains a major challenge. A simple straightforward hypothesis may involve the modulation of pH-dependent protein synthesis, in other words by blocking NHE-1 activity, the resultant intracellular acidotic environment will result in reduced protein synthesis and hence, reduced hypertrophy. However, this concept may not be viable, primarily because it is likely that despite NHE-1 inhibition, other intracellular pH-regulatory processes would likely be recruited to assure intracellular pH homeostasis. Thus, in view of the multiplicity of intracellular pH-regulatory mechanisms in the cardiac cell, it is doubtful that intracellular acidosis would be markedly greater in hearts treated with NHE-1 inhibitors, although this needs to be determined with certainty. However, it should be noted that in acute ischaemia experiments when NHE-1 is inhibited, intracellular pH does not fall lower than values seen in the absence of NHE-1 blockade [30] or, if intracellular pH is reduced, the reduction does not occur until late in the ischaemic period [31].

A potentially interesting hypothesis which could represent the underlying basis for the role of NHE-1 in hypertrophy/heart failure involves sodium ions which are important mediators of cell hypertrophy [32,33]. Accordingly, the accompanying reduction in sodium entry occurring during NHE-1 inhibition may represent the major basis for salutary effects of NHE-1 inhibition on hypertrophy and heart failure. Indeed, in a recent study which utilised neonatal rat ventricular myocytes exposed to hypertrophic agents it was proposed that NHE-1-dependent sodium influx is a major contributor to hypertrophy produced by these factors including α_1-adrenoceptor agonists, ET-1, or phorbol ester and which involved sodium-induced activation of PKC isoforms, especially PKCδ and PKCε [33]. Indeed, inhibitors of PKC were found to reduce the hypertrophic response whereas the NHE-1 inhibitor, HOE-694, decreased both the hypertrophy as well as PKC activation [33], thus reinforcing this link between PKC and NHE-1. However, other cell signalling mechanisms may also participate. For example, stretch-induced cardiac cell hypertrophy was also associated with Raf-1 and MAPK activation, both of which were blocked by HOE-694 [22]. Taken together, these findings are suggestive of NHE-1 involvement in the activation of various kinases resulting in cell growth [22], although the role of NHE-1 as a direct influence on the hypertrophic process cannot be ruled out with certainty. It is also likely that the overall role of NHE-1 in this process reflects a multiplicity of mechanisms acting in concert to produce the resultant phenotypic change. A summary of these concepts is presented in **Figure 2**.

Figure 3: The effect of a cariporide-containing diet on *in vivo* haemodynamic characteristics in rats three months after myocardial infarction produced by coronary artery ligation (CAL). Animals were divided according to the degree of infarct sizes.
LVSP: left ventricular systolic pressure; LVEDP: LV end-diastolic pressure; LV $+dP/dt_{max}$: LV maximal increase in pressure over time; LV dP/dt_{max}: LV maximal decrease in pressure over time. $^{*}p < 0.05$, $^{**}p < 0.01$ from respective sham values. Percentages in parentheses indicate the mean percent change from sham. Numbers in parentheses at the bottom depict the number of animals in each group. With permission of the American Physiological Society [28].



6. Summary and expert opinion

Although this area of investigation is still relatively new, the preceding discussion is suggestive of an important role of NHE-1 inhibitors for the treatment of hypertrophy and heart failure and offers the potential for these agents to reduce the maladaptive myocardial responses which culminate in heart failure. It is possible in fact that the mechanistic basis for various pharmacological agents which are used today for treating heart failure such as ACE inhibitors or which are undergoing clinical evaluation (i.e., endothelin receptor antagonists), reflects in part NHE-1 inhibition. A major difference, however, between these agents and specific NHE-1 inhibitors is that the latter are devoid of direct vascular or haemodynamic influence and produce beneficial effects independently of preload or afterload inhibition. As such they offer potential as adjunctive therapy to produce additive or synergistic actions. Much work needs to be done to understand the mechanism for NHE-1 involvement, particularly with respect to the remodelling response. Various other questions need to be addressed, some of which have been touched upon in this review. For example, do these agents produce additive or synergistic actions in combination with other therapeutic strategies for heart failure? Can NHE-1 inhibitors reverse the hypertrophic response once it has been initiated? Do NHE-1 inhibitors attenuate hypertrophy and heart failure produced by other factors? Although many issues need further studies, experimental evidence is encouraging for the emergence of NHE-1 inhibition as a therapeutic strategy for the effective treatment of heart failure.

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